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COMPARATIVE ANTI-PHLOGISTIC ACTIVITY OF Δ^9-TETRAHYDROCANNABINOL, HYDROCORTISONE AND ASPIRIN IN VARIOUS RAT PAW EDEMA MODELS

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Summary

The anti-edema activity of THC has been directly compared to that of hydrocortisone and aspirin in rat paw edemas produced by eleven different phlogistic agents. A significant effective dose for THC could only be achieved in carrageenan, dextran, formalin, kaolin and Na urate induced edemas, while hydrocortisone and aspirin were active against all edemas except that produced by serotonin. Based on these data it is suggested that the anti-edema effects of THC most likely occur by a different mode of action than either the classic steroid or non-steroidal anti-inflammatory agents.

Recent reports from our laboratories have shown that Δ^9-tetrahydrocannabinol (THC), which is reported to be the major psychoactive constituent of marihuana (1), and other naturally occurring cannabinoids possess significant anti-edema activity following oral administration in the rat carrageenan edema and adjuvant arthritis tests (2,3). The results of the above *in vivo* experiments taken together with the findings of Burstein and Raz (4) and Burstein, *et al.* (5) that these substances markedly inhibit the biosynthesis of prostaglandin E_2 *in vitro* suggest a possible causal relationship similar to what has been postulated by Vane (6) as a mechanism of action for the non-steroidal anti-inflammatory agents aspirin and indomethacin.

The purpose of the following investigation was to further

explore the anti-edema activity of THC by determining its effect against several phlogistic agents used to produce rat paw edema. In each experiment THC was directly compared with hydrocortisone and aspirin.

Materials and Methods

Non-fasted, Charles River male CD rats (Sprague-Dawley strain) weighing 100 to 120 grams at the time of testing were used in this investigation only after an acclimation period of at least four days to the laboratory environment had elapsed.

All drugs and their vehicles were administered by the oral route. THC was prepared in undiluted propylene glycol for administration in such a way as to permit a volume of 0.1 ml per 100 grams of body weight. Hydrocortisone and aspirin were suspended in 1% gum acacia in a concentration such that 0.5 ml per 100 grams of body weight was given to each rat. Control rats received an equal volume of vehicle.

One hour following oral vehicle or drug administration rats (N = 6 per dose of drug) received an injection of one of the following phlogistic agents (7-10) just under the plantar surface of the right hind paw: 0.05% bradykinin (free base, 0.1 ml), 1% carrageenan (0.05 ml), 1% dextran (0.05 ml), fresh undiluted egg white (0.05 ml), 1% formalin (0.1 ml), 0.1% histamine (free base, 0.1 ml), 10% kaolin (0.05 ml), 2.5% mustard (0.1 ml), 0.02% serotonin (free base, 0.05 ml), 10% Na urate crystal suspension (0.2 ml) and 2.5% brewer's yeast (0.1 ml). Volume of the injected paw to the level of the

lateral malleolus was measured by water displacement immediately before oral administration and again 30 minutes after the injection of bradykinin or histamine, 45 minutes after serotonin injection, two hours after injection of dextran, brewer's yeast or egg white, three hours after carrageenan, four hours following formalin or mustard and five hours after kaolin and Na urate. The difference between the two measurements was designated edema volume.

Drug effectiveness was analyzed in the following manner. Mean (± S.E.) paw volumes, pre- and post-drug, were statistically compared using the Student's t test. The percent edema inhibition was obtained from the ratio of the pre-drug volume : post-drug volume. In addition, the number of rats at each dose level whose edema volume was at least 25 percent less than that of the mean for the vehicle-treated control group was also tallied. An ED_{50} value was then calculated based on this all-or-none representation of the data (11).

Results

Table 1 lists oral ED_{50} values obtained for THC, hydrocortisone and aspirin against the eleven different phlogistic agents used in these studies. THC proved effective against only five phlogistic agents and resulted in the following order of sensitivity: carrageenan (2.3 mg/kg) > Na urate (16.2 mg/kg) > kaolin (19.5 mg/kg) > dextran (32.0 mg/kg) > formalin (53.0 mg/kg). Following administration of the maximum dose of THC used (80.0 mg/kg) rat paw edemas induced by the remaining

TABLE 1

Oral ED_{50} Values for THC, Hydrocortisone and Aspirin Against Rat Paw Edema Produced by Various Phlogistic Agents

Edema-Inducing Substance	Oral ED_{50}, mg/kg (± 95% Confidence Limits)[a]		
	THC	Hydrocortisone	Aspirin
Bradykinin	>80.0	48.5(25.5-92.3)	255.0(134.0-485.0)
Carrageenan	2.3(1.3-4.2)	2.2(1.2-4.0)	50.0(31.3-80.0)
Dextran	32.0(21.6-47.4)	13.7(7.5-25.1)	340.0(230.0-505.0)
Egg White	>80.0	13.6(7.5-24.8)	200.0(129.0-310.0)
Formalin	53.0(42.0-66.8)	0.8(0.5-1.3)	18.0(9.9-32.4)
Histamine	>80.0	17.0(7.1-40.8)	34.5(18.9-63.2)
Kaolin	19.5(9.3-40.7)	0.3(0.1-0.6)	38.0(20.2-71.5)
Mustard	>80.0	5.2(2.5-11.0)	100.0(63.0-159.0)
Serotonin	>80.0	>80.0	>400.0
Na Urate	16.2(6.6-39.8)	5.6(3.3-9.5)	38.5(21.0-70.5)
Yeast	>80.0	6.9(3.8-12.5)	182.0(102.0-326.0)

[a] That dose of drug producing a 25% or greater inhibition of edema formation in 50% of the animals tested.

six phlogistins were not significantly reduced. On the other hand, hydrocortisone and aspirin significantly reduced edema formation caused by all phlogistic agents with the exception of serotonin. In addition to the number of phlogistic agents sensitive to THC and hydrocortisone or aspirin there are differences in mg/kg potency between these agents. For instance, the rank order of potency against dextran, kaolin and Na urate edemas was hydrocortisone > THC > aspirin, while in formalin edema it was hydrocortisone > aspirin > THC. On the other hand, THC and hydrocortisone being equally effective against carrageenan edema were far superior to aspirin.

FIG. 1 presents a summary of the anti-edema activity of each drug in the form of a profile of activity. The mean percent inhibition for each drug at a common dose used against each phlogistic agent as determined by the dose response and ED_{50} data listed in Table 1 is presented. THC at 20.0 mg/kg significantly reduced only carrageenan, kaolin and Na urate edemas. On the other hand, 20.0 mg/kg of hydrocortisone significantly inhibited all edemas except that produced by bradykinin and serotonin, while aspirin (200.0 mg/kg) was ineffective against one additional phlogistin, i.e., dextran. Although the anti-phlogistic profiles of aspirin and hydrocortisone are remarkably similar, magnitude of effect and mg/kg potency are in favor of the latter agent. Percent inhibition of those edemas significantly affected by each drug differed considerably. Against carrageenan edema hydrocortisone, THC and

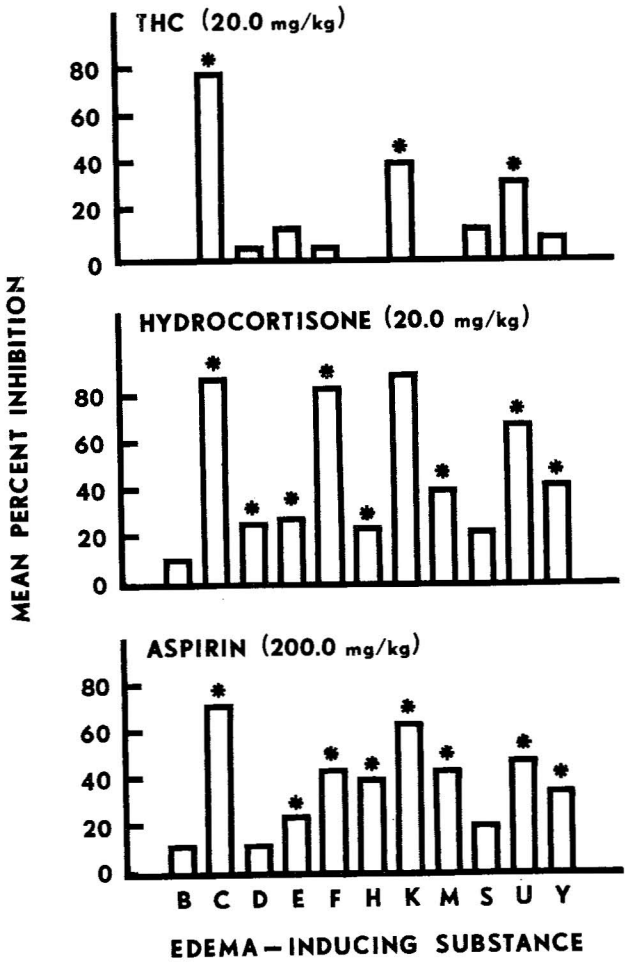

FIG. 1

Anti-phlogistic Profiles of THC, Hydrocortisone and Aspirin (B - bradykinin, C - carrageenan, D - dextran, E - egg white, F - formalin, H - histamine, K - kaolin, M - mustard, S - serotonin, U - Na urate and Y - brewer's yeast; *$p \leq 0.05$).

aspirin produced 88, 77 and 66 percent reductions, respectively. On the other hand, kaolin edema was reduced 90 percent by hydrocortisone, 64 percent by aspirin and only 39 percent by THC. Likewise, Na urate was most sensitive to hydrocortisone (67 percent inhibition) and moderately affected by aspirin (48 percent inhibition).

Discussion

The comparative anti-edema effect of THC, hydrocortisone and aspirin was studied against inflammation in the rat paw produced by eleven different phlogistic agents. THC exhibited a different profile of activity against these various types of rat paw edema than either hydrocortisone or aspirin. ED_{50} values for THC could be obtained only against carrageenan, dextran, formalin, kaolin and Na urate, while effective doses of hydrocortisone and aspirin could be achieved against all phlogistins except serotonin.

Inflammation has been shown to be a relatively complex and non-specific response to a wide variety of noxious stimuli (7) including those phlogistic agents used in this study. There is considerable evidence which indicates the release of certain substances such as histamine, serotonin, bradykinin and prostaglandins (12) at various stages of the inflammatory response. Hence, insight to a possible mode of action for an anti-inflammatory agent may be achieved by determining its anti-phlogistic profile (7) as described in this investigation. Since the results of the present investigation show that the

anti-phlogistic profile for THC differs substantially from hydrocortisone and aspirin, it seems reasonable to speculate that its mode of action is also different. Additional experiments must be conducted which clearly define the role of each mediator (12) at each stage of the inflammatory response. Not until the effect each steroidal (hydrocortisone) and non-steroidal (aspirin) anti-inflammatory agent as well as THC might have on these mediators, can a rational explanation be drawn as to the mechanism(s) of action of any of these agents.

Because of the more recent findings of Vane (13) the prostaglandins have been implicated as mediators of not only inflammation, but also of fever and pain. Sofia, et al. (2) have shown that THC does possess potent analgesic effects, but lacks antipyretic activity. The biosynthesis of prostaglandins is a multi-stage process and various inhibitors can act at different points (14). In addition, Vane (13) points out that prostaglandin synthetases from various tissues have remarkably different sensitivities to various drugs. Hence, these two factors may account for differences seen among drugs, including THC, as to their degree of anti-inflammatory, analgesic or antipyretic activity.

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